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Endosphaera engelmanni Endoparasitic in *Trichodina spheroidesi*
Infecting the Puffer, *Sphæroides maculatus*.

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(Plates I & II; Text-figures 1 & 2).

INTRODUCTION.

Endosphaera engelmanni Entz is a suctionian parasite of both marine and fresh-water protozoans, especially those belonging to the Order Peritricha. However, many investigators usually associate this interesting parasite with a fresh-water protozoan host, giving only passing reference to its marine occurrence (see Doflein, 1928; Kudo, 1946).

Among the *Trichodina*, *T. pediculus* commonly found on *Hydra* was reported as being infected with *E. engelmanni* by Sand (1899). There is some evidence indicating that the "larval" stage described and figured by Chatton (1910) for *Amoeba mucicola* endoparasitic in *Trichodina labrorum* (from the gills of European marine fishes) may be a stage in the development of *Endosphaera engelmanni*.

Lynch and Noble (1931) have given the best description of *Endosphaera engelmanni*, which they found parasitic in the fresh-water peritrich, *Opisthonecta henneyi*. The present studies deal with the presence of this parasite in a marine species of *Trichodina* and will compare the morphology, cytology and life history of this marine endoparasite with its fresh-water relatives.

MATERIAL AND METHODS.

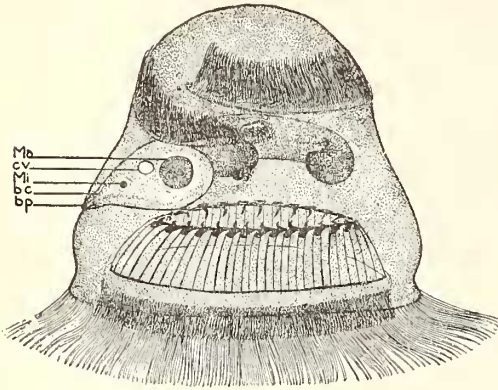
During the 1938 epizootic trichodiniasis among puffers, *Sphæroides maculatus* (Bloch & Schneider), in littoral waters of New York and New Jersey, the two gill parasites responsible, *Trichodina spheroidesi* Padnos & Nigrelli and *T. halli* Padnos and Nigrelli, were found to be infected with *Endosphaera engelmanni*. The incidence of infection was 20% among the former and 2% among the latter species of *Trichodina*. This study was made only on preserved material which was fixed in Schaudinn's fluid and 10% formalin. The Schaudinn-treated material was stained with iron hematoxylin; the formalin material was impregnated with 5% silver nitrate solution.

THE PARASITE.

The young form of the parasite, including the free living stage (swarmer) as well as the bud within the parent cell, measures from 9 to 16 microns in diameter (average diameter 11 microns), is round or slightly ovoid in shape and contains a rounded macronucleus 3-8 microns in diameter (average, 6 microns) and a micronucleus, 0.5-1.5 microns in diameter (average, 1 micron). A contractile (?) vacuole is present which is most clearly defined in specimens identified as recently emerged forms and in fully developed buds within the parent cell (Text-fig. 2, E & F). These measurements are in close agreement with those given by Lynch and Noble (1931) for corresponding stages of their fresh-water form. These investigators also reported three rows of equatorial cilia on the newly formed bud and the free-swimming swimmers. Only 0.1% of the parasites observed in our preparations were swimmers. In the hematoxylin-stained individuals the ciliary pattern could not be determined. In the silver nitrate-treated material, however, equatorially arranged granules were seen in the fully developed buds still within their parent cells (Text-fig. 2, E). These were placed so closely that their number and arrangement could not be determined. Since the measurements and cytological characters of the parasite in the present study closely correspond to those described by Lynch and Noble (1931), we do not hesitate to assign it to the same species, *Endosphaera engelmanni* Entz.

Endosphaera engelmanni enters the host by penetrating its pellicle, to which it remains attached by a short stalk through which passes a canal terminating in a birth pore (Text-fig. 1). The macronucleus is central in position in the free end of the organism, while the micronucleus is situated some distance away towards the narrower attached end.

Through study of a number of parasitic stages, it appears that reproductive activity (endogenous budding) is initiated by a swelling process. Measurements made on parasites in varying growth stages prior to the formation of the bud, indicate that the cell diameter increases to twice its original size (12-30 microns, average 21 microns). The diameter of the ovoid-shaped macronucleus is also doubled (4-11 microns, average 9 microns). (See Plate I, Figs. A-D.) These measurements correspond closely to those given by Lynch and Noble (1931) for parasites showing no traces of buds.



TEXT-FIG. 1. *Trichodina spheroidesi* parasitized by *Endosphaera engelmanni*. Side view. $\times 950$. Semi-diagrammatic reconstruction from iron hematoxylin and silver nitrate preparations. Ma, macronucleus; cv, contractile (?) vacuole; Mi, micronucleus; bc, birth canal; bp, birth pore.

The preliminary swelling of the macronucleus is followed by a further expansion during which it becomes irregular in shape (Plates I & II, Figs. E-G). During this period of macronuclear swelling, the micronucleus starts mitotic activity and develops its metaphase. At this stage, the cytoplasmic area around the micronucleus is denser and stains more deeply with hematoxylin than the rest of the cell cytoplasm. This area is the anlage of the cytoplasmic substance of the bud (Plate I, Fig. D).

With continued growth the macronucleus extends part of its mass into the denser cytoplasm (Plate I, Figs. E, F) where it is pinched off to become the macronucleus of the bud (Plate II, Figs. G-J). During this period the micronucleus passes into telophase and divides into two. One micronucleus remains within the newly formed cytoplasmic region while the other migrates out of this field and becomes associated with the parent macronucleus (Plates I & II, Figs. F-I). Such newly formed buds average 10 microns in diameter; their macronuclei average 5 microns and micronuclei 1 micron. The

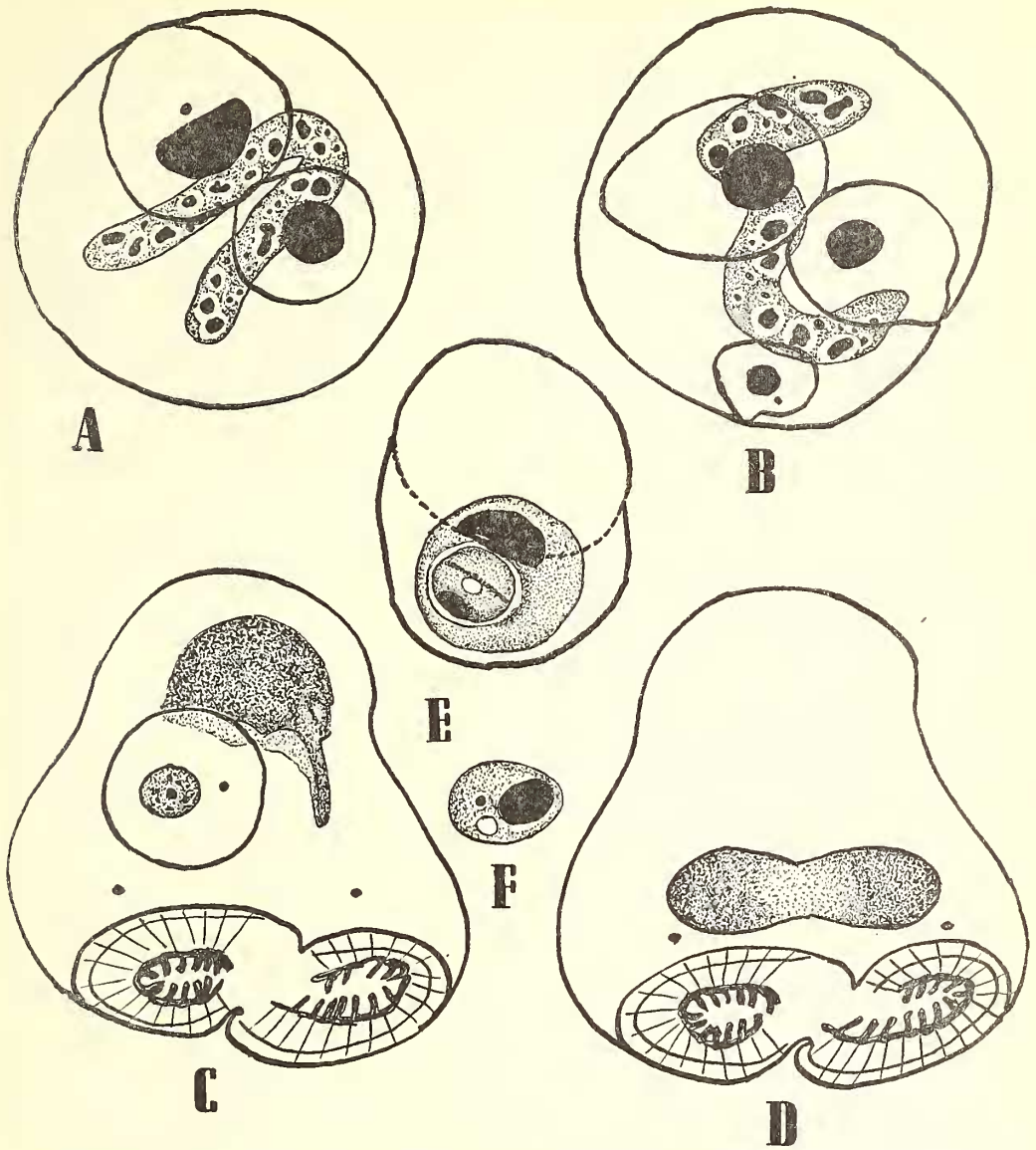
cytoplasmic area, now at its greatest density, develops a membrane around its periphery, becomes distinct and separates from the parent cytoplasm and lies in a space at the base of the birth canal (Plate II, Figs. H-K). The mature bud or swarmer probably passes into the birth canal and out through the birth pore to become a free-swimming organism. Myonemes in the vicinity of the birth pore, as described by Lynch and Noble (1931), were not present in our preparations.

Although as many as three parasites were observed in one *Trichodina*, in no case was more than one bud found in the reproducing individuals. However, we agree with Lynch and Noble (1931) that the parent cell continues to produce more buds, since in no case have we observed evidence of disintegration of the parasite associated with this phase of development.

EFFECTS OF THE PARASITE ON THE HOST.

Endosphaera engelmanni in *Trichodina* apparently interferes with the normal development of the host by exerting pressure on the host's cytoplasm and macronucleus. In many of the single infections and in all of the double and triple ones, the host macronucleus is displaced and distorted. An extreme malfunctioning of the host's macronucleus under such a pressure was noted in parasitized *Trichodina* undergoing binary fission. Under such conditions, the macronucleus of the host was forced into the upper third of the cell and therefore was prevented from participating in the fission process, resulting in its failure to pull apart (Text-fig. 2, C). The normal position of the macronucleus in non-parasitized *Trichodina* at this stage of fission is in the lower two-thirds of the organism (Text-fig. 2, D). The normal trophic macronucleus of *Trichodina* is horse-shoe shaped. Many examples of distortion of the macronucleus were observed, especially in double and triple infections (Text-fig. 2, A & B).

Another sign of disturbance of the host's macronucleus, probably resulting from the presence of the growing parasite, is seen in the internal changes of that structure. In the normal macronucleus, chromatin granules are homogeneously distributed throughout the matrix, whereas the macronucleus of the parasitized protozoan contains large chromatin clumps within vacuolated areas (Text-fig. 2, A & B). These macronuclear changes apparently occur when the parasite occupies about half of the volume of the host. This is indicative of macronuclear disintegration, ending in death of the host. Similar results were described by Sassuchin (1934) for the ciliate, *Nyctotherus ovalis*, infected with a fungus, particularly when the sporangium of the parasite occupied a large volume of the host.



TEXT-FIG. 2. Effects of *Endosphaera engelmanni* on *Trichodina*. $\times 950$. From iron-hematoxylin preparations. **A**, double infection. Note the distortion of the host macronucleus, vacuolated areas in the macronuclear substance. **B**, triple infection. **C**, single infection with displacement of host macronucleus during fission. **D**, normal position of macronucleus during fission in non-parasitized *Trichodina*. **E**, Stage showing completely formed bud. Silver nitrate preparation. $\times 950$. Note equatorial band of basal granules and contractile (?) vacuole. **F**, free-living form (swarmer) of the parasite. Hematoxylin preparation. $\times 950$. Cilia are not evident with this preparation.

SUMMARY AND CONCLUSIONS.

1. *Trichodina spheroidesi* and *T. halli*, parasites on the gills of the puffer, *Sphaeroides maculatus*, were found infected with a suctionian parasite belonging to the genus *Endosphaera*.

2. The nuclear behavior and the formation of the bud is described. The anlage of the bud is recognized as early as the metaphase

stage of the micronucleus of the parent cell. This is manifest as a concentration of denser cytoplasm around the dividing micronucleus.

3. The development of cilia could not be followed in detail, but bands of basal granules around the equatorial region of the bud were recognized. No evidence of myoneme structures in the vicinity of the birth pore were observed.

4. Mechanical and physiological disturbances to the host's cytoplasm and macronucleus are apparent with the growth of the parasite. Distortion and displacement of the macronucleus as well as disintegration of the macronuclear substances was observed.

5. The suctorian is in all probability *Endosphaera engelmanni* Entz, previously described from fresh-water protozoa, since morphological and cytological studies reveal no recognizable differences.

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EXPLANATION OF THE PLATES.

Endosphaera engelmanni parasitic in *Trichodina spheroidesi*. Camera lucida drawings of hematoxylin-stained specimens. $\times 950$.

PLATE I.

- Fig. A. Initial penetration by *Endosphaera*.
Fig. B. Double infection. Early growth stage of the parasite. Note swelling of the macronucleus.
Fig. C. Double infection. Continued swelling of the macronucleus.
Fig. D. Micronucleus in metaphase surrounded by densely staining cytoplasmic area.
Fig. E. Irregularly shaped macronucleus with cytoplasmic area more concentrated. Micronucleus in teleophase.
Fig. F. Micronucleus in late telophase.

PLATE II.

- Fig. G. Start of the process of endogenous budding. Part of the macronucleus extends into the newly formed cytoplasmic area. Micronucleus divided. One micronucleus remains within cytoplasmic area; the other becomes associated with the parent macronucleus.
Fig. H. A later stage of the process shown in G. The macronucleus resembles a mushroom. The cytoplasm of the bud is separated and distinct from the surrounding cytoplasm.
Figs. I & J. Macronuclear material pinched off into cytoplasmic area.
Figs. K & L. Fully formed bud in parent cell.